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How Bugs Make Lassos

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In this issue of *Chemistry & Biology*, Knappe et al. report the generation of a series of mutants of the bacterial peptide capistruin to help unravel key aspects of the biosynthesis of its unusual lasso structure.

Lasso peptides are a structurally unique family of ribosomally synthesized natural products produced by certain bacteria to defend against competing strains (Rebuffat et al., 2004; Severinov et al., 2007). They are characterized by an 8- or 9-residue macrolactam ring formed through the crosslinking of their N-terminal amino group with the side-chain carboxyl of a glutamic- or aspartic-acid residue. Remarkably, the remaining C-terminal tail segment is threaded through this ring, resulting in the lasso-like arrangement that has given the family its name. Unfolding of this threaded structure is prevented by bulky amino acids on both sides of the ring, making the fold exceptionally stable. So far, six peptides have been found to adopt such a structure and they have been divided into class I and class II lasso peptides based on the presence or absence of two disulfide bonds, respectively, in addition to the threaded noose.

Capistruin is a 19-residue class II lasso peptide encoded by the *capABCD* gene cluster in the pathogenic bacterium *Burkholderia thailandensis* (Knappe et al., 2008). It was discovered through genome mining studies that identified homology between each of the four genes in the *capABCD* cluster and the corresponding four plasmid encoded genes *mcjABCD,* which are responsible for producing the related microcin J25 in *Escherichia coli*. The cluster encodes the 47 residue precursor protein CapA, two processing enzymes, CapB and CapC, which are responsible for the maturation of the CapA precursor, and CapD, which is homologous to ABC transporters and required for export and self-immunity. CapB and CapC are weakly homologous to bacterial transglutaminase and asparagine synthetase B, respectively. It is proposed that during capistruin maturation, CapB cleaves the CapA precursor to free the Gly1 N terminus, and CapC activates the side-chain carboxyl group of Asp9 to promote the condensation reaction. The tight threading of the C-terminal tail through the macrolactam ring in mature capistruin means the CapA precursor has to be folded correctly beforehand, so that the ring is enclosed around the C-terminal tail. Mature capistruin has potent antimicrobial activity on *Burkholderia* and *Pseudomonas* strains that are closely related to *B. thailandensis*.

In the featured article, the authors systematically modified the CapA precursor to decipher the requirements for it to interact with, and be folded and secreted by the CapB, CapC, and CapD system. Using an elegantly designed expression system in *E. coli,* they made 48 mutated variants of the precursor protein and investigated their ability to be processed into mature capistruin (Knappe et al., 2009). The mutations included an Ala-scan of all residues in the mature domain and the proximal residues upstream in the prosequence, as well as variants probing the implications of extending or truncating the C-terminal tail or the N-terminal ring. Strikingly, the capistruin sequence was readily amenable to changes, with most variants folding correctly. Exceptions included a tri-peptide region (Arg11-Val12-Ile13) near the critical aspartic acid at position 9 and a single residue in the prodomain, Thr27, which are presumably critical for recognition by the CapB and CapC processing enzymes. Changes to the N-terminal ring, either by the addition or the deletion of a residue, or by changing the critical cross-linked Asp9 to a Glu, are not tolerated, and all such variants do not fold correctly. In contrast, the C-terminal tail can be extended by one residue, or truncated by up to three residues, without disruption to the folding. Furthermore, double and triple mutations of bulky residues in the C-terminal segments were produced to investigate the role of Arg15, Phe16, and Phe18 in preventing the unfolding of the precursor. Interestingly, Arg15 was found to be the key residue for keeping the C-terminal tail trapped. The finding that only a small subset of amino acids is required for correct folding is consistent with studies on microcin J25; however, for microcin J25, key residues are located in the N-terminal ring and the threaded segment rather than the loop (Pavlova et al., 2008).

The lasso peptides share some features with another family of peptides having a complex topology, namely the cyclotides from plants (Craik, 2006). Cyclotides have their N and C termini linked by a peptide bond, creating a seamless peptide backbone with no beginning or end. During the initial characterization of a lasso peptide microcin J25, it was mistakenly believed to be a head-to-tail cyclic peptide like the cyclotides, in part because it could be enzymatically digested without dissociation into two peptide fragments (Rebuffat et al., 2004). It was subsequently shown that this is because the N-terminal ring in microcin J25 wraps so tightly around the C-terminal tail that the fragments can not be separated under standard analytical conditions (Rosengren et al., 2003; Bayro et al., 2003; Wilson et al., 2003). Thus the initial comparison with cyclotides was flawed, but when the lasso structure was revealed, so was a similarity in threading. In addition to their cyclic backbone, cyclotides contain a cystine knot in which two disulfide bonds and the interconnecting backbone form an embedded ring that is threaded by a third disulfide bond. Figure 1 shows a comparison of the cores of microcin J25, capistruin, and the prototypic cyclotide kalata B1.

What makes these complex peptides so interesting? Naturally, their interlocked structures are intriguing in their own right and raise questions about the mechanisms of folding and processing that are nicely addressed in the study by Knappe et al. (2009). But the most striking common feature of these peptides is their unique chemical and biological stability. Lassos and cyclotides are extremely resistant to proteolytic degradation or denaturation by heat, and in many ways behave more like small organic molecules than proteins, thus raising the possibility that the poor stability typically associated with peptide-based drug leads might be overcome using these novel topologies (Clark et al., 2005). The characterization of the requirements for correct folding of capistruin by Knappe et al. (2009) demonstrates that there is scope for new modifications of the peptide sequence to optimize its antibiotic spectrum or, we believe, to introduce epitopes with new bioactivities. This paves the way for future exploration of capistruin and other lasso peptides as templates for drug development. The noose that bacteria use to strangle their competitors might thus turn out to be a beneficial rope trick in pharmaceutical design.

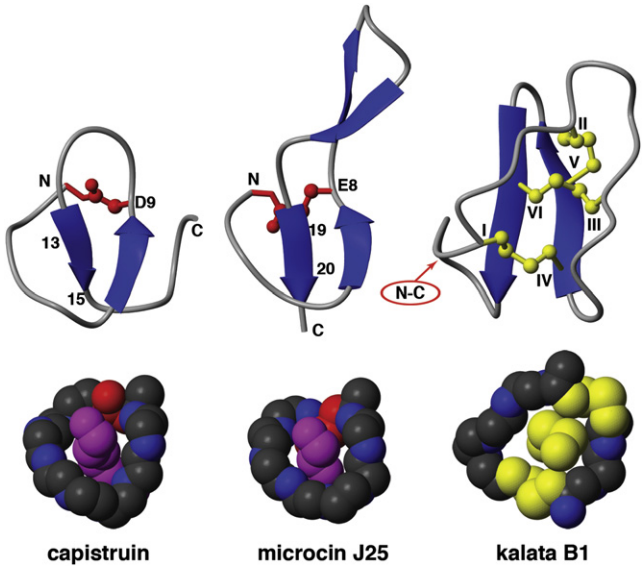

Figure 1. Threaded and Knotted Peptides
The lasso peptides capistruin and microcin J25 from bacteria, and the cyclotide kalata B1 from plants, share similar features in that the small β sheets are stabilized by topologically restricting covalent crosslinks to form ultra-stable structures (upper panel). A key feature of all of these peptides is a tightly threaded embedded ring (lower panel); in the lasso peptides, the peptide backbone itself passes through the ring, whereas in the cyclotides a disulfide bond threads the ring to form a cystine knot. The cyclotides have the additional feature of a head-to-tail cyclised backbone (ligation point indicated by an arrow).

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